Effect of Corn Stover Hydrolysate and Temperature on Fermentation Performance of Selected Yeast Strains

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Abstract

Technoeconomic analysis of a biomass to ethanol process shows a 7.7% cost savings of ethanol per gallon using a fermentative organism tolerant to acid-pretreated biomass hydrolysate and elevated temperatures. The cost saving is translated from the reduction of contamination and subsequent product loss and the elimination of hydrolysate detoxification. An evaluation was conducted to examine the effect of neutralized corn stover hydrolysate and elevated temperatures on the fermentation performance of various wild-type yeast strains. Twenty four strains were selected from literature and screened for fermentation performance in the presence of 0%, 25%, 50%, 75%, and 90% (v/v) hydrolysate concentration. The same strains were tested at temperatures of 30ºC, 35ºC, 40ºC, and 42ºC. While many strains achieved high ethanol yields in 90%(v/v) hydrolysate, ethanol productivity decreased by 50-70% indicating toxicity of the hydrolysate on cell growth and fermentation. Ethanol productivity rapidly decreases as fermentation temperature increased up to 42ºC. Four strains demonstrated higher tolerance to temperature compared to the other strains performance. Two strains, *Candida acidothermophilum* #20381 and *Saccharomyces cerevisiae* #26602, demonstrated the best fermentation performance in both conditions.

Introduction

The robustness of a microorganism in a lignocellulosic biomass to ethanol fermentation can be beneficial to the economics of the process. Acid-pretreated biomass hydrolysate has been shown to contain toxic compounds at various levels, depending on the feedstock, that inhibit growth and fermentation of the organism. The most commonly reported compounds are acetic acid, furfural, lignin degradation products, and 5-hydroxymethylfurfural. Methods such as overliming, ion-exchange chromatography, solvent extraction, and treatment with activated carbon have shown to reduce toxicity of the hydrolysate, however, the addition of one or more of these process steps adds to the cost of producing ethanol from these materials.

In addition to hydrolysate tolerance, a strain tolerant to elevated temperatures can be beneficial to the process by operating at fermentation conditions that limit contamination. Sugar loss from contaminating organisms adds to the cost by reducing ethanol yields. Additionally, elevated temperatures benefit enzymatic hydrolysis of cellulose in simultaneous saccharification and fermentation (SSF) processes.

Twenty four wild-type yeast strains were selected from literature and evaluated in fermentations with acid-pretreated corn stover hydrolysate neutralized to fermentation pH and in fermentations with a temperature range from 30ºC to 42ºC.

Materials and Methods

Microorganisms:
Freeze-dried cultures were streaked for isolation at 30ºC on YM agar plates (Difco). Isolates were cultured twice at 30ºC in fermentation media (CYA) consisting of 1% clarified corn steep liquor (Golden Technologies, Johnstown, CO), 0.05% yeast extract (Difco), 0.25% ammonium sulfate (Sigma), 2% glucose (Sigma), and 0.025M sodium citrate (pH 5.8). Cultures concentrated and mixed with 25%(v/v) glycerol and stored at -80ºC.

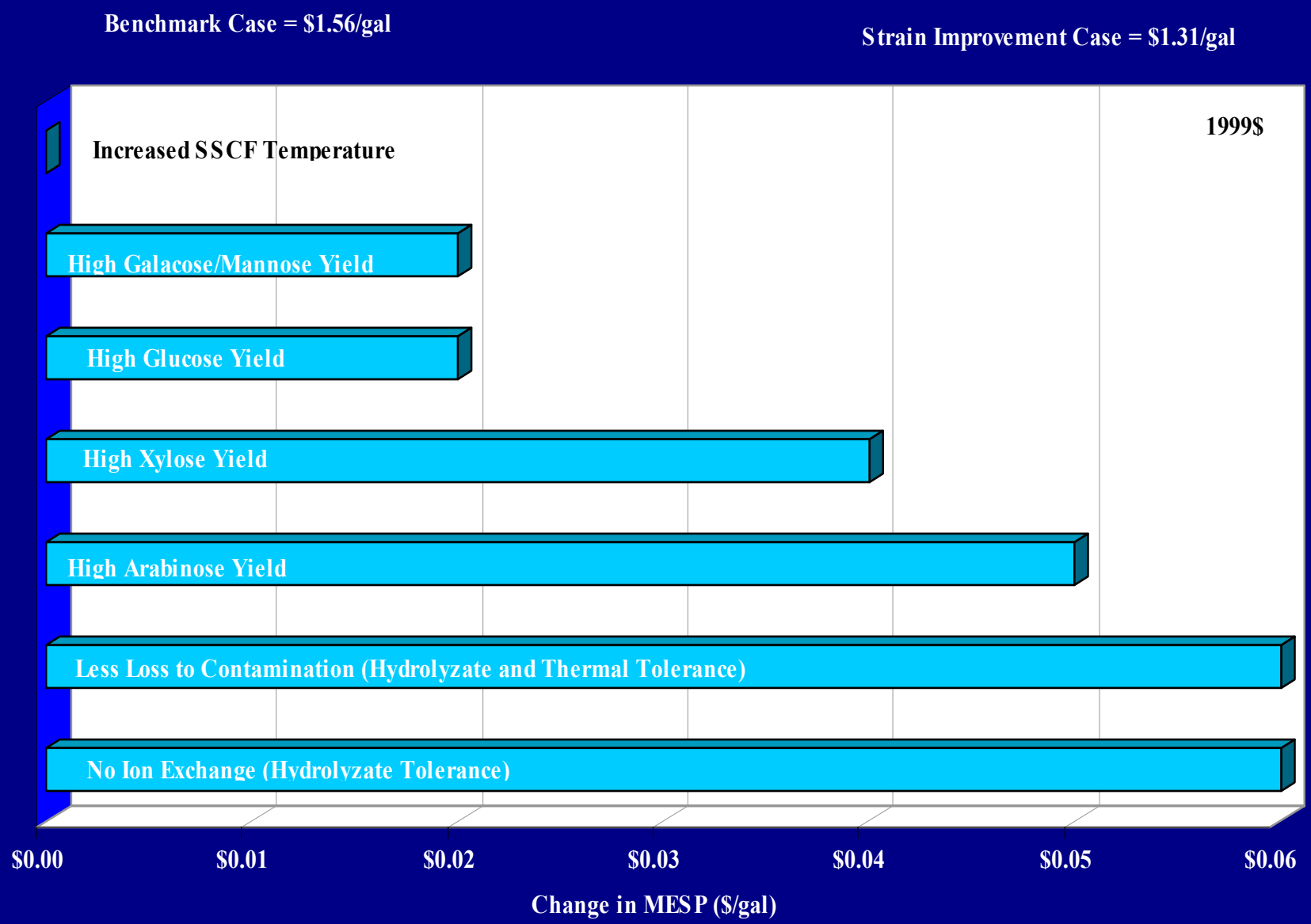
Inoculum:
Frozen stocks used to inoculate seed flasks at a starting OD_{600} = 0.05. Cultures shaken at 30ºC, 180 rpm for 14 hours.

Hydrolysate fermentations:
Corn stover was pretreated at 165ºC for 8 min. in 0.6%(w/w) sulfuric acid at 20% dry solids. Hydrolysate separated by centrifugation. Hydrolysate composition: glucose 6g/l, xylose 37.4g/l, galactose 3.1g/l, arabinose 6.6g/l, mannose 2.9g/l, acetic acid 4.3g/l, HMF 0.17g/l, and furfural 1.6g/l. Hydrolysate neutralized to pH 6.0 with sodium hydroxide pellets and 0.22um filter sterilized. Yeast strains inoculated at OD_{600}=0.1 in flasks with 0%, 25%, 50%, 75%, and 90%(v/v) concentrations of hydrolysate supplemented with CYA (no glucose) medium. Glucose was added to a final concentration of 20g/l for all hydrolysate levels. Flasks shaken at 180 rpm, 30ºC, for 24 to 48 hours.

Temperature fermentations:
Strains inoculated into CYA medium with 20 g/l glucose at a starting OD_{600}=0.1 and shaken at 180 rpm, for 24 hours in 30ºC, 35ºC, 40ºC, and 42ºC.

Sample analysis:
Growth was measured turbidometrically at 600 nm (1-cm light path (Milton Roy Spectronic 601)). Cell-free culture broth was analyzed for sugars, ethanol, and organic acids by HPLC (Hewlett Packard Series 1090L with HP1047A refractive index detector, Wilmington, DE). An organic acid column (Bio-Rad HPX-87H, Richmond, CA) was used at 65ºC with 0.01N H2SO4 mobile phase at a flow rate of 0.6 ml/minute.

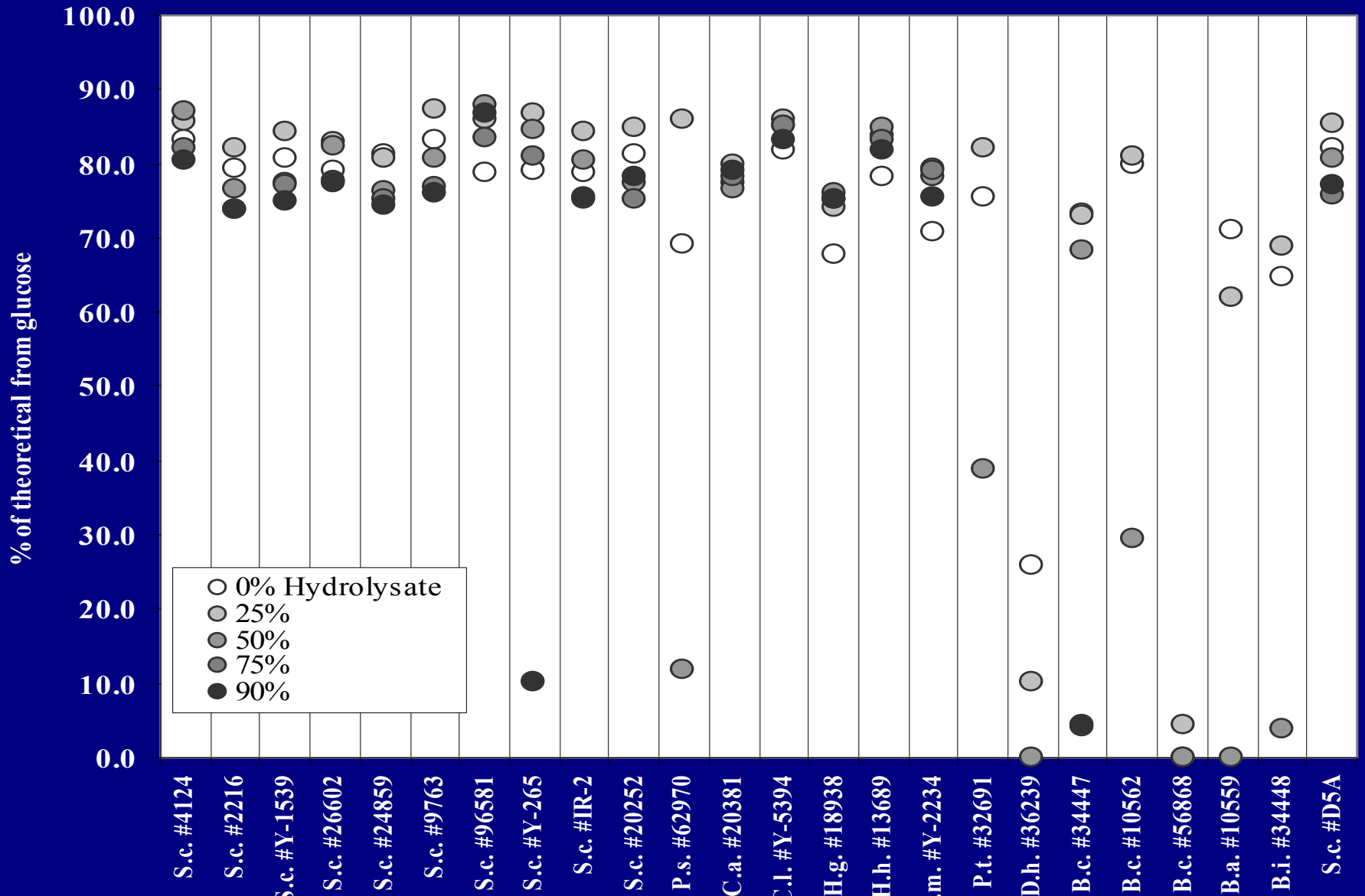
Technoeconomic Analysis of Strain Selection



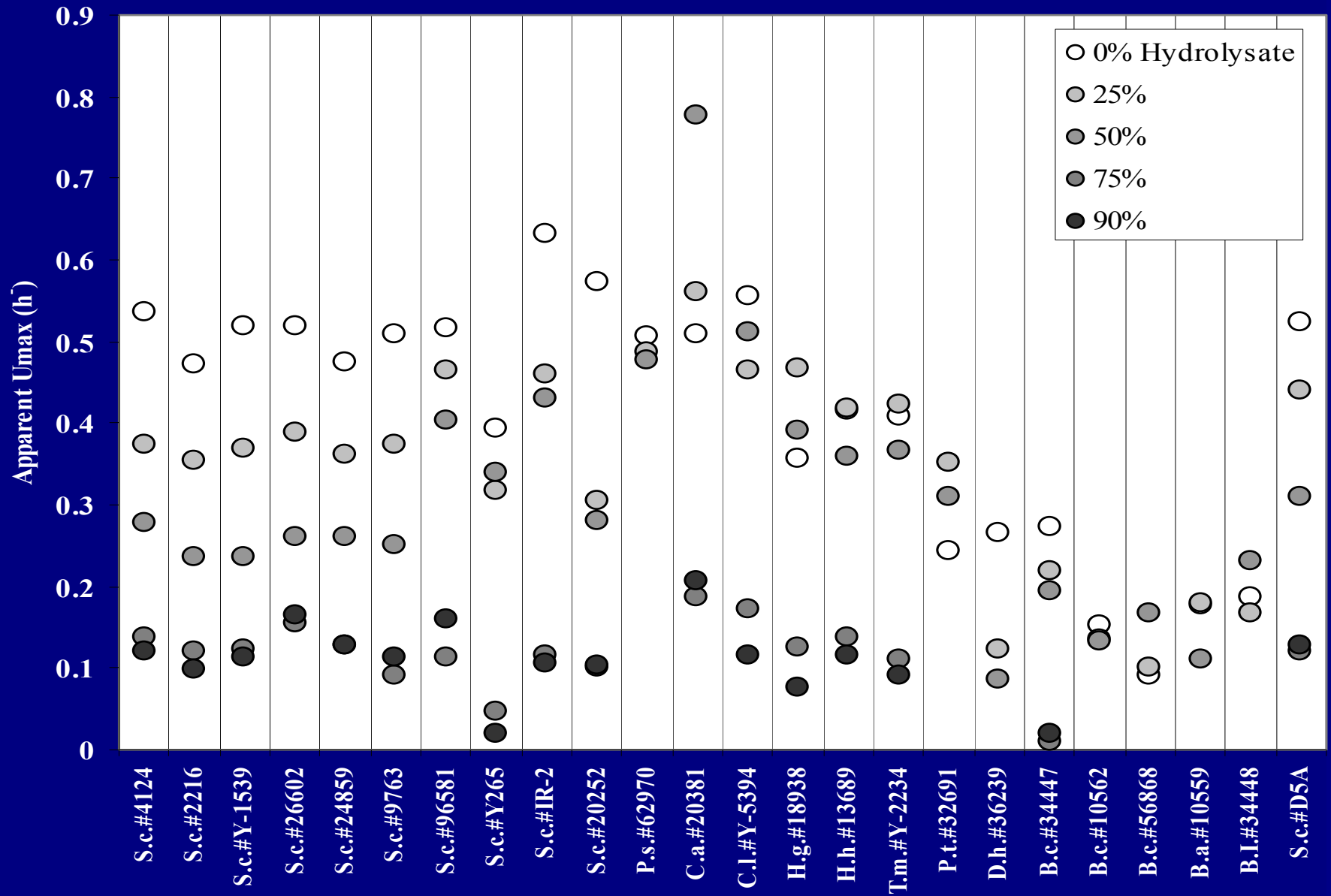
List of Strains

Strain		Description	Reference
Saccharomyces cerevisiae	ATCC #4124	High ethanol	McDermott 1984
	JCM #2216	High ethanol	Yamada et al. 1951
	NRRL #Y1539	High ethanol	Yamada et al. 1951
	ATCC #26602	High ethanol	Zayed 1997
	ATCC #24859	Biomass fermentation	Kunduru and Pometto 1996
	ATCC #9763	High ethanol	Ziffer and Iosif 1982
	ATCC #96581	Hydrolysate fermentation	Palmqvist et al. 1999
	NRRL #Y265	Flocculating	Hojo et al. 1999
	NEDO #IR-2	Flocculating	Ohashi et al. 1998
	ATCC #20252	Temperature and ethanol tolerant	Slapack et al. 1987
Pichia stipitis	ATCC #62970	Ferments xylose	Kotter et al 1993
Candida acidothermophilum	ATCC #20381	Temperature tolerant	Kadam et al. 1997
C. lusitaniae	NRRL #Y5394	Ferments cellobiose	Spindler et al. 1992
Hansenula glucozyma	ATCC #18938	Ferments cellobiose	Spindler et al. 1992
H. holstii	ATCC #13689	Ferments cellobiose	Spindler et al. 1992
Torulopsis molischiana	NRRL #Y2234	Ferments cellobiose	Spindler et al. 1992
Pachysolen tannophilus	ATCC #32691	Ferments xylose	Ktuse et al. 1996
Debaryomyces hansenii	ATCC #36239	Hydrolysate fermentation	Cruz et al. 2000
Brettanomyces custersii	ATCC #34447	Ferments cellobiose	Spindler et al. 1992
B. clausenii	ATCC #10562	Ferments cellobiose	Spindler et al. 1992
B. clausenii	ATCC #56868	Ferments cellobiose	Spindler et al. 1992
B. anomalus	ATCC #10559	Ferments cellobiose	Spindler et al. 1992
B. intermedia	ATCC #34448	Ferments cellobiose	Spindler et al. 1992
S. cerevisiae	NREL #D5A	Biomass fermentation	Hatzis et al. 1996

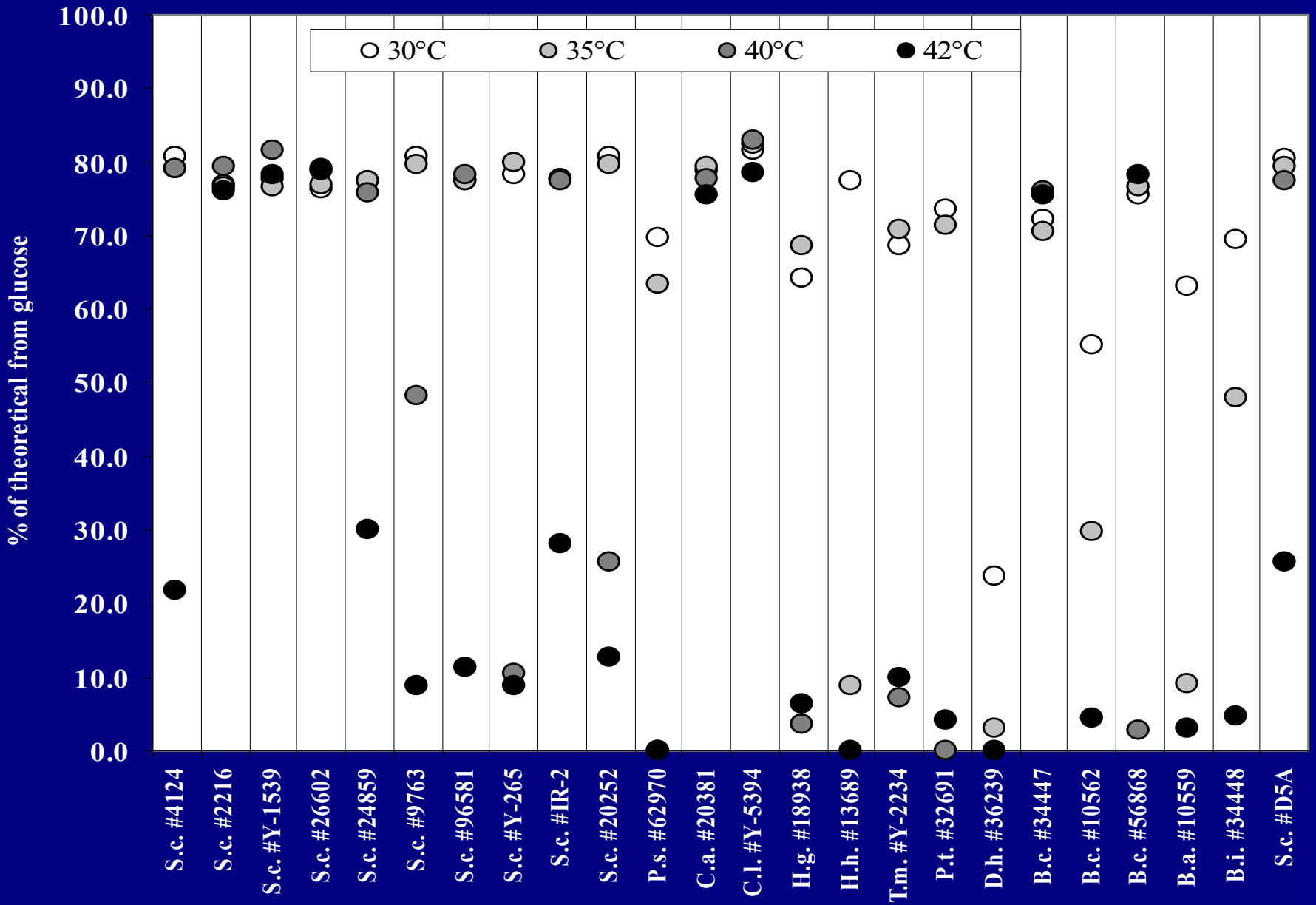
Theoretical Ethanol Yield from Glucose at Varying Concentrations of Corn Stover Hydrolysate



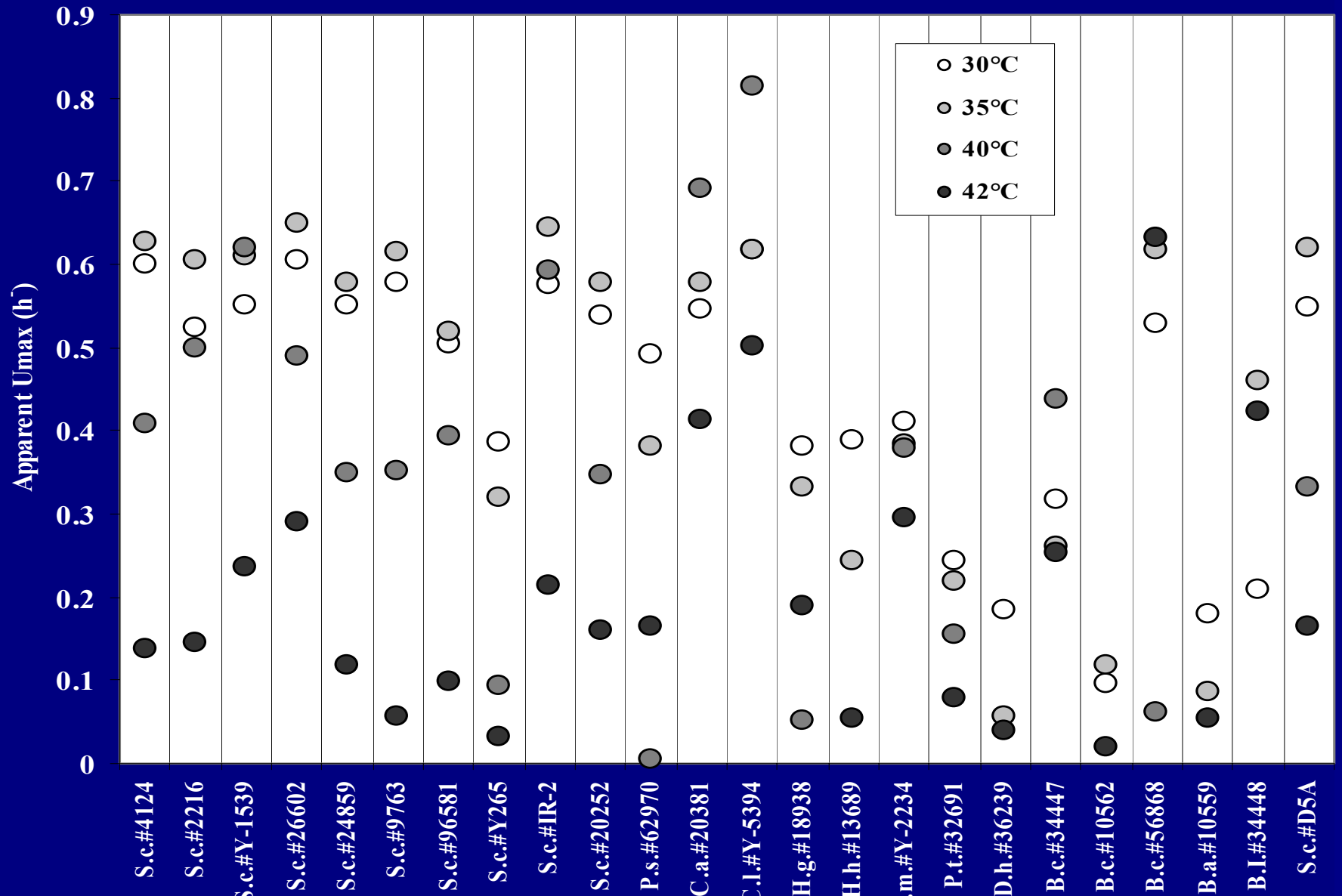
Maximum Specific Growth Rate at Varying Concentrations of Corn Stover Hydrolysate



Theoretical Ethanol Yield at Varying Temperatures



Maximum Specific Growth Rate at Varying Temperatures



Conclusions

- 15 of 24 strains tested show similar maximum ethanol yields at all levels of hydrolysate.

- Hydrolysate toxicity was better observed by maximum specific growth rate and ethanol productivity where inhibition is observed at hydrolysate concentrations as low as 25%(v/v).

- At 90%(v/v) hydrolysate, *Candida acidothermophilum* #20381 demonstrates the greatest tolerance based on the highest early-stage ethanol productivity. Five *Saccharomyces cerevisiae* strains demonstrated higher mid-stage ethanol productivity.

- 6 of 24 strains show similar maximum ethanol yields at all fermentation temperatures tested.

- Observation of early, mid, and late ethanol productivity values show several strain have increased productivity at 35°C compared to 30°C. Ethanol productivity decreases at 40°C and 42°C.

- Two *Saccharomyces cerevisiae* (#Y-1539 and #26602) and two *Candida* (#20381 and Y-5394) have the highest ethanol productivity values at 42°C.

- *Candida acidothermophilum* #20381 and *Saccharomyces cerevisiae* #26602 demonstrated higher tolerance to both hydrolysate and temperature.

Acknowledgement

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